

WHAT IS CLAIMED IS:

1. A DNA segment comprising an isolated coding region that encodes a substantially full length P-TEFb subunit, wherein the coding region is characterized as:

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- (a) encoding a substantially full length P-TEFb kinase subunit having the amino acid sequence of SEQ ID NO:2; or
- (b) encoding a substantially full length P-TEFb large subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50; or as a substantially full length coding region that hybridizes to the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:43 or SEQ ID NO:48 under stringent hybridization conditions.
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2. The DNA segment of claim 1, wherein said isolated coding region encodes a substantially full length P-TEFb kinase subunit having the amino acid sequence of SEQ ID NO:2.

3. The DNA segment of claim 2, wherein said isolated coding region has the nucleotide sequence from position 115 to position 1327 of SEQ ID NO:1.

4. The DNA segment of claim 1, wherein said isolated coding region encodes a substantially full length P-TEFb large subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50; or is a substantially full length coding region that hybridizes to the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:43 or SEQ ID NO:48 under stringent hybridization conditions.

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5. The DNA segment of claim 1, wherein said isolated coding region encodes a substantially full length P-TEFb large subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50 and wherein said coding region hybridizes to the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:43 or SEQ ID NO:48 under stringent hybridization conditions.

6. The DNA segment of claim 4, wherein said isolated coding region encodes a substantially full length P-TEFb large subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50.

7. The DNA segment of claim 6, wherein said isolated coding region encodes a P-TEFb large subunit having the amino acid sequence of SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50.

8. The DNA segment of claim 7, wherein said isolated coding region encodes a P-TEFb large subunit having the amino acid sequence of SEQ ID NO:45.

9. The DNA segment of claim 7, wherein said isolated coding region encodes a P-TEFb large subunit having the amino acid sequence of SEQ ID NO:47.

10. The DNA segment of claim 7, wherein said isolated coding region encodes a P-TEFb large subunit having the amino acid sequence of SEQ ID NO:50.

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11. The DNA segment of claim 4, wherein said isolated coding region is a substantially full length coding region that hybridizes to the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:43 or SEQ ID NO:48 under stringent hybridization conditions.

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12. The DNA segment of claim 11, wherein said isolated coding region has the nucleotide sequence of SEQ ID NO:44.

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13. The DNA segment of claim 11, wherein said isolated coding region has the nucleotide sequence of SEQ ID NO:46.

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14. The DNA segment of claim 11, wherein said isolated coding region has the nucleotide sequence of SEQ ID NO:49.

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15. The DNA segment of claim 1, wherein said DNA segment comprises a first coding region that encodes a substantially full length P-TEFb kinase subunit and a second coding region that encodes a substantially full length P-TEFb large subunit.

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16. The DNA segment of claim 15, wherein said second coding region encodes a P-TEFb large subunit that has the amino acid sequence of SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50.

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17. The DNA segment of claim 16, wherein said first coding region encodes a P-TEFb kinase subunit that has the amino acid sequence of SEQ ID NO:6.

18. The DNA segment of claim 16, wherein said second coding region has the nucleotide sequence of SEQ ID NO:44, SEQ ID NO:46 or SEQ ID NO:49, and wherein said first coding region has the nucleotide sequence of SEQ ID NO:5.

19. The DNA segment of claim 1, wherein said isolated coding region is operatively attached to a second coding region that encodes a selected peptide or protein sequence, said DNA segment encoding a P-TEFb subunit fusion protein.

20. The DNA segment of claim 1, operatively positioned under the control of a promoter.

21. The DNA segment of claim 20, further defined as a recombinant vector.

22. The DNA segment of claim 20, comprised within a recombinant host cell.

23. An expression system comprising:

- (a) a first expression unit comprising, under the transcriptional control of a promoter, a first coding region that encodes a substantially full length P-TEFb kinase subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:2 or SEQ ID NO:6; and

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- (b) a second expression unit comprising, under the transcriptional control of a promoter, a second coding region that encodes a substantially full length P-TFb large subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50.

24. The expression system of claim 23, wherein said first and said second expression units are comprised on a single expression vector.

25. The expression system of claim 23, wherein said first and said second expression units are comprised on two distinct expression vectors.

26. The expression system of claim 23, wherein said expression system is comprised within a recombinant host cell.

27. A recombinant host cell comprising at least a first DNA segment in accordance with claim 1.

28. The recombinant host cell of claim 27, wherein said cell is a prokaryotic host cell.

29. The recombinant host cell of claim 27, wherein said cell is a eukaryotic host cell.

30. The recombinant host cell of claim 27, wherein said cell further comprises an HIV Tat protein.

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31. The recombinant host cell of claim 27, wherein said cell comprises a first DNA segment that encodes a substantially full length P-TEFb kinase subunit and a second DNA segment that encodes a substantially full length P-TEFb large subunit.

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32. The recombinant host cell of claim 31, wherein said first and second DNA segments are comprised within a single expression vector.

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33. A method for detecting P-TEFb nucleic acids in a sample, comprising obtaining sample nucleic acids from a sample suspected of containing P-TEFb nucleic acids; contacting said sample nucleic acids with a nucleic acid segment that hybridizes to the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:43 or SEQ ID NO:48, under conditions effective to allow hybridization of substantially complementary nucleic acids; and detecting the hybridized complementary nucleic acids thus formed.

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34. The method of claim 33, wherein the sample nucleic acids are obtained from a sample suspected of containing a tumor cell.

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35. A method of using a DNA segment that encodes a substantially full length P-TEFb subunit, comprising expressing at least a first DNA segment in accordance with claim 1 in a recombinant host cell and collecting the P-TEFb subunit expressed by said cell.

36. A composition comprising at least a first isolated, substantially full length P-TEFb protein subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50.

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37. The composition of claim 36, wherein said substantially full length P-TEFb subunit is operatively attached to a selected peptide or protein sequence.

10 38. The composition of claim 36, wherein said composition comprises a substantially full length P-TEFb kinase subunit in operative association with a substantially full length P-TEFb large subunit.

15 39. The composition of claim 36, further comprising an HIV Tat protein.

20 40. An isolated, functional P-TEFb/enzyme complex comprising a P-TEFb kinase subunit in operative association with a P-TEFb large subunit.

25 41. A P-TEFb immunodetection reagent characterized as:

(a) an antibody that has immunospecificity for a P-TEFb subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:47 or SEQ ID NO:50; or

(b) an antibody that has immunospecificity for a P-TEFb subunit that includes a contiguous sequence of at least about 7 amino acids from SEQ ID NO:6, the antibody operatively attached to a detectable label.

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42. A method of identifying a gene that encodes a protein that interacts with a P-TEFb subunit, comprising the steps of:

- (a) obtaining a first DNA segment comprising a candidate gene; said first DNA segment expressing a first fusion protein comprising a transcriptional transactivating domain operatively attached to the candidate protein encoded by said candidate gene;
- (b) obtaining a second DNA segment that expresses a second fusion protein comprising a P-TEFb subunit operatively attached to a DNA binding domain that binds to a defined nucleic acid sequence;
- (c) providing said first and second DNA segments to a eukaryotic host cell that comprises a marker gene operatively positioned downstream of said defined nucleic acid sequence; and
- (d) identifying a eukaryotic host cell that expresses said marker gene, thereby identifying said candidate gene as a gene that encodes a protein that interacts with a P-TEFb subunit.

43. A method for identifying a candidate transcriptional inhibitor, comprising preparing a P-TEFb composition comprising at least a P-TEFb kinase subunit and testing said candidate inhibitor for the ability to inhibit P-TEFb-mediated phosphorylation of RNA polymerase II, wherein inhibition of phosphorylation is indicative of a candidate transcriptional inhibitor.

44. The method of claim 43, comprising the steps of:

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- (a) obtaining a P-TEFb composition comprising at least a P-TEFb kinase subunit;
- (b) obtaining an RNA polymerase II composition comprising at least the carboxyl terminal domain (CTD) of the large subunit of RNA polymerase II;
- (c) admixing said P-TEFb composition with said RNA polymerase II composition and an effective phosphate donor compound comprising a labeled phosphate group; and
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- (d) determining the ability of said P-TEFb composition to transfer said labeled phosphate group to said RNA polymerase II composition in the presence of said candidate transcriptional inhibitor and in the absence of said candidate transcriptional inhibitor, wherein a reduction in the amount of labeled phosphate transferred to RNA polymerase II in the presence of said candidate is indicative of a candidate transcriptional inhibitor
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20 45. The method of claim 43, wherein said P-TEFb composition comprises a P-TEFb enzyme complex that has transcription elongation promoting activity.

25 46. The method of claim 45, further comprising testing the candidate transcriptional inhibitor so identified in a transcription elongation assay, wherein inhibition of transcription elongation confirms the identification of a transcriptional inhibitor.

30 47. The method of claim 43, further comprising the step of purifying the candidate transcriptional inhibitor so identified.

48. A method for identifying an HIV Tat protein, comprising contacting a composition suspected of containing an HIV Tat protein with a human P-TEFb composition under conditions effective to allow the formation of bound protein complexes and detecting the bound Tat:P-TEFb protein complexes so formed.

49. A method for identifying a candidate viral transcription inhibitor, comprising testing a candidate substance for the ability to inhibit the binding of a viral transcriptional transactivator protein to at least one human P-TEFb subunit under effective binding conditions, wherein inhibition of binding is indicative of a candidate viral transcription inhibitor.

50. The method of claim 49, wherein said viral transcriptional transactivator protein or said at least one human P-TEFb subunit is attached to a solid support.

51. The method of claim 49, wherein said effective binding conditions comprise admixing a human cell nuclear extract with said viral transcriptional transactivator protein and said at least one human P-TEFb subunit.

52. The method of claim 49, wherein said inhibition of binding is tested by determining the ability of the candidate substance to inhibit the formation of viral protein-P-TEFb subunit complexes that are detected by means of a detectable label attached to the viral protein or to the P-TEFb subunit.

53. The method of claim 49, wherein said inhibition of binding is tested by determining the ability of the candidate substance to inhibit the formation of viral protein-P-TEFb subunit complexes that are detected by means of a first specific immunological detection reagent.

54. The method of claim 53, wherein said inhibition of binding is tested by determining the ability of the candidate substance to inhibit the formation of viral protein-P-TEFb subunit complexes that are detected by means of a first and a second specific immunological detection reagent.

55. The method of claim 49, further comprising testing the candidate viral transcription inhibitor so identified in a viral transcription elongation assay, wherein inhibition of viral transcription elongation confirms the identity of an active candidate viral transcription inhibitor.

56. The method of claim 55, further comprising testing the active candidate viral transcription inhibitor next identified in separate human and viral transcription elongation assays, wherein inhibition of viral transcription elongation, and not human transcription elongation, in the presence of said viral transcriptional transactivator protein confirms the identification of an active viral transcription inhibitor.

57. The method of claim 49, further comprising the step of purifying the candidate viral transcriptional inhibitor so identified.

58. The method of claim 57, wherein said purified candidate viral transcriptional inhibitor is formulated in a pharmaceutically acceptable vehicle.

59. A method for identifying a candidate viral transcription inhibitor, comprising testing a candidate substance for the ability to inhibit viral RNA elongation in a functional viral transcription elongation assay, wherein inhibition of viral RNA elongation is indicative of an active candidate viral transcription inhibitor.

60. The method of claim 59, further comprising testing the active candidate viral transcription inhibitor in distinct human and viral transcription elongation assays, wherein the inhibition of viral, but not human, transcription elongation confirms the identity of an active candidate viral transcription inhibitor.

61. The method of claim 60, wherein said method comprises the steps of:

- (a) preparing a first transcriptionally competent composition capable of generating elongated human RNA transcripts, said composition comprising effective amounts of human nucleic acid template, P-TEFb enzyme complex, RNA polymerase II, the required nucleotide triphosphates and ATP;
- (b) preparing a second transcriptionally competent composition capable of generating elongated viral RNA transcripts, said composition comprising effective amounts of viral nucleic acid template, viral transcriptional transactivator protein, P-TEFb enzyme complex, RNA polymerase II, the required nucleotide triphosphates and ATP; and
- (c) identifying a viral transcription inhibitor that inhibits the generation of elongated viral RNA transcripts by said second transcriptionally competent composition but that does not inhibit the generation of elongated human RNA transcripts by said first transcriptionally competent composition.

62. The method of claim 61, wherein said candidate viral transcription inhibitor is a candidate HIV transcription inhibitor and wherein said method comprises the steps of:

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- (a) preparing a first transcriptionally competent composition capable of generating elongated human RNA transcripts, said composition comprising effective amounts of human nucleic acid template, P-TEFb enzyme complex, RNA polymerase II, the required nucleotide triphosphates and ATP;
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- (b) preparing a second transcriptionally competent composition capable of generating elongated HIV RNA transcripts, said composition comprising effective amounts of HIV nucleic acid template, HIV Tat protein, P-TEFb enzyme complex, RNA polymerase II, the required nucleotide triphosphates and ATP; and
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- (c) identifying an HIV inhibitor that inhibits the generation of elongated HIV RNA transcripts by said second transcriptionally competent composition but that does not inhibit the generation of elongated human RNA transcripts by said first transcriptionally competent composition.
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63. The method of claim 59, further comprising the step of purifying the candidate viral transcriptional inhibitor so identified.

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64. The method of claim 63, wherein said purified candidate viral transcriptional inhibitor is formulated in a pharmaceutically acceptable vehicle.

65. A method for inhibiting viral replication, comprising contacting a cell suspected of being infected with a virus with a biologically effective amount of a viral transcription inhibitor identified by the method of claim 59.

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66. The method of claim 65, wherein said cell is a cell suspected of being infected with HIV.

67. The method of claim 65, wherein said cell is located within an animal and a
10 therapeutically effective amount of said inhibitor is administered to said animal.

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